Oral methionine loading as a cause of acute serum folate deficiency: its relevance to parenteral nutrition

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Summary  
Oral methionine supplements (8-0 g daily for 4 days) were given to five normal volunteers who continued to eat their usual diet. This treatment resulted in a significant fall in serum folate concentration. Three days after the end of treatment concentrations had not completely returned to control values. The fall in concentration was prevented by giving oral folic acid supplements. It is suggested that folic acid supplements should be given to patients who are receiving intravenous infusions of amino acid mixtures.

Introduction  
Acute serum folate deficiency as a result of parenteral nutrition was first reported by Wardrop et al. (1975), who suggested that the ethanol infused in their patients might have been wholly or partially responsible. However, two cases who had not received ethanol have since been reported (Green, 1977). It was therefore possible that other constituents of the nutritional regimens might affect folate metabolism.

<table>
<thead>
<tr>
<th>TABLE 1. Methionine content of some commercially available preparations containing amino acids. The values are taken from manufacturers' data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
</tr>
<tr>
<td>-------------------------------</td>
</tr>
<tr>
<td>'Aminosol 10%'</td>
</tr>
<tr>
<td>'Vamin' preparations</td>
</tr>
<tr>
<td>'Aminoplex 5'</td>
</tr>
<tr>
<td>'Aminoplex 14'</td>
</tr>
<tr>
<td>'Aminofusin L 600' and 'L 1000'</td>
</tr>
<tr>
<td>'Synthamin 9'</td>
</tr>
<tr>
<td>'Synthamin 14'</td>
</tr>
<tr>
<td>'Synthamin 17'</td>
</tr>
</tbody>
</table>

Methionine, which is an important source of one carbon (1-C) units, is a major constituent of all the commercially available amino acid preparations (Table 1). Folate derivatives are involved as co-factors in normal 1-C unit utilization and thus an excess of methionine might influence folate metabolism.

Subjects and methods  
Five healthy volunteers gave informed consent to participate in the study. Three were male and two female, and the age range was 20–31 years. Control samples were taken on day 1. Methionine (Koch-Light Laboratories Ltd.) 2 g q.d.s. was given orally in water for 4 days starting immediately after the control blood sample had been taken. This dose was chosen because it is similar to that given by Wardrop et al. (1975). Blood samples (20 cm³) were taken at 12.00 noon each day until the methionine was stopped on day 5. In three subjects samples were also taken on day 8. All the volunteers ate their usual diet throughout the course of the study.

Two volunteers were studied again after an interval of 6 weeks, when an identical protocol was followed except that they were given an oral supplement of folic acid 5 mg daily during the period of methionine administration.

All estimations were performed daily. Haemoglobin, packed cell volume (PCV), mean corpuscular volume (MCV), red cell count (RBC), white cell count (WBC) and platelet count were measured on a 'Haemalog 8' counter, and blood films examined. Serum and red blood cell folate (RBC–folate) were assayed biologically using Lactobacillus casei (var. rhamnosus). When methionine was added to serum to give a concentration of 300 μmol/l it did not affect the folate assay. Methionine was assayed in deproteinized plasma using a 'Chromaspek' amino acid auto-analyser. Analysis of the amino acids in

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Table 2. Haematological changes during methionine loading. Control samples were taken on day 1. Results are given as mean ± s.e. mean for five subjects (four subjects only on day 5)

<table>
<thead>
<tr>
<th>Day</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC 10¹²/l</td>
<td>5·25 ± 0·10</td>
<td>5·31 ± 0·06</td>
<td>5·48 ± 0·11</td>
<td>4·96 ± 0·04*</td>
<td>4·86 ± 0·23</td>
</tr>
<tr>
<td>PCV %</td>
<td>44·1 ± 0·6</td>
<td>44·3 ± 0·5</td>
<td>45·4 ± 0·9</td>
<td>43·6 ± 1·0</td>
<td>45·9 ± 2·1</td>
</tr>
<tr>
<td>MCV fl</td>
<td>82·2 ± 2·1</td>
<td>83·6 ± 1·6</td>
<td>83·0 ± 1·8</td>
<td>88·0 ± 1·3†</td>
<td>90·3 ± 3·6</td>
</tr>
<tr>
<td>WBC 10⁹/l</td>
<td>5·5 ± 0·7</td>
<td>6·1 ± 0·8</td>
<td>6·7 ± 1·0</td>
<td>6·3 ± 0·8</td>
<td>6·9 ± 1·0*</td>
</tr>
</tbody>
</table>

Results significantly different from day 1: * P < 0·05; † P < 0·025.

The preparation of methionine ingested by the volunteers showed it to contain 98·3% methionine and 1·7% histidine.

Results are expressed as the mean ± s.e. mean and significance values are derived from the paired t test (single tailed).

Results

Serum folate concentrations dropped significantly 24 hr after starting methionine supplements (Fig. 1), and remained low throughout the treatment period. In the three subjects in whom serum folate concentration was measured three days after stopping methionine the concentration was still lower (4±1 ± 1·0 μg/l) than their control value (5·5 ± 0·8 μg/l). No change was seen in the red cell folate concentrations (Fig. 1). In the two subjects in whom the study was repeated with oral folate supplements the serum folate concentration rose progressively (Fig. 2).

No changes occurred in haemoglobin concentration, platelet count or PCV. No macrocytes were seen in the blood films and there was no reticulocytosis, but MCV rose and RBC fell during treatment, both changes being statistically significant on day 4 (Table 2). There was an increase in WBC which was statistically significant on day 5 (Table 2).

Plasma methionine concentrations (Fig. 1) rose from normal values before treatment to an average of 181 μmol/l (range 102–341) during methionine supplementation. Three days after the end of treatment methionine concentrations had returned to within normal limits.

Discussion

This study has shown that the serum folate concentration falls acutely when normal subjects eating their usual diet are given a supplement of methionine. The conditions of this study differ from those during intravenous feeding in at least two ways. Firstly, the methionine was given orally. However, the resulting plasma levels were comparable to those previously reported in patients receiving 7·2 g of methionine daily as part of their intravenous regimen (Smits and Wells, 1975). Secondly, the subjects in the present study continued to eat their usual diet, and thus adequate folate was available to allow normal metabolism of dietary methionine. It is suggested that by giving the methionine supplement the normal relationship between methionine and folate has been disturbed in a similar way to that
which occurs in patients given equivalent amounts of intravenous methionine but no folate.

The fall in serum folate concentration recorded here was smaller than that reported by Wardrop et al. (1975). This difference is probably due to the impoverished stores and total lack of folate intake in their patients. Red cell folate concentrations were normal in the index patients of Wardrop et al. (1975), and remained normal in the present study.

There is no clear explanation for the fall in RBC and rises in MCV and WBC which occurred in this study. The changes were most marked on days 4 and 5 and it is unlikely that these changes could have originated from an effect of methionine on the bone marrow because they appeared so rapidly. The rise in MCV occurred without any reticulocytosis or visual evidence of macrocytosis in the blood films. It is possible that the rise in MCV may be more apparent than real because the figure for MCV obtained from the Haemalog 8 counter is calculated from RBC and PCV so that a constant PCV despite a fall in red cell count, as occurred in this study, would result in a high calculated MCV. However, it is difficult to explain how the PCV could remain constant as RBC fell, unless there were a real increase in red cell size.

This study gives no information on the exact mechanism by which methionine supplements alter folate metabolism. Methionine loading will probably increase the proportion of methyltetrahydrofolic acid (methyl-THFA) and other reduced folates in serum (Krebs and Hems, 1976). Although such a change might not directly affect serum folate activity as measured by L. casei, it could result in a redistribution of folate from serum to cells because reduced folates are only weakly bound to serum folate binding protein and are therefore more available for cellular uptake (Waxman, 1975). A redistribution of folate from serum to cells could cause a relative lack of folate in some tissues. Folate concentrations in cerebrospinal fluid, for example, are normally three times those in serum because methyl-THFA is actively transported into the choroid plexus (Taguchi et al. 1977).

Whatever the pathogenesis may be, treatment with 5 mg of folic acid daily was more than adequate to prevent the fall in serum folate concentration in normal subjects. The minimal daily requirement of folic acid in an adult is 0.05–0.1 mg (Herbert, 1962). Wretlind (1974) has recommended that this should be doubled in patients on intravenous feeding. However, even this may be inadequate when methionine is given to patients whose stores of folate may be depleted. Until more definite information on folate requirements in these patients is available it is suggested that an initial dose of 10 mg to replenish the body store, followed by 0.5 mg daily be given.

Patients other than those on parenteral nutrition are also given methionine supplements. On the evidence from this study it would probably be advisable to give folic acid to patients who are treated with methionine after paracetamol overdose.

Methionine is only one of several substrates used in parenteral nutrition which can serve as sources of 1–C units. Other amino acids which might contribute to folate depletion by this means include histidine (via the formimino group of formimino-glutamic acid) and serine (Harper, 1973). Another donor of 1–C units is choline which is present in the phosphatides of soya oil emulsion; 1 litre/day of soya oil emulsion 10% contains more choline than the normal daily intake (Wretlind, 1972).

Acknowledgments
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References
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